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Stability and reliability of biological reactors

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Dynamac Corporation

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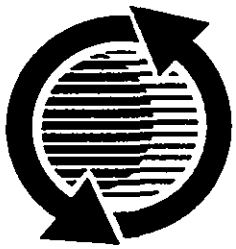

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Stability and reliability of biological reactors

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ABSTRACT

Bioregenerative resource recovery components for Advanced Life Support systems will need to be reliable and stable for long duration space travel. Since 1989, bioregenerative life support research at the ALS Breadboard Project has examined processing of inedible crop residues in bioreactors for recovery of nutrients for replenishment of crop hydroponic solutions. Bioreactor operation has been reliable as demonstrated by continuous operation for up to 418 days with long periods of steady state conditions. Bioreactors have demonstrated stability following unplanned, non-lethal perturbations in pH, temperature, dissolved oxygen, and inedible residue supply. In each instance, a rapid return to steady state conditions was observed.

INTRODUCTION

Five fundamental attributes can be used to compare ALS hardware and processes: reliability, manpower/automation, mass, energy, and volume. Reliability is the most important requirement of a long-term regenerative life support system. The operational life support scenario will be long-term, remote human habitation in an extremely hostile environment. Access to spare parts and commodities such as oxygen, water, and carbon, will be limited to periodic and partial resupply. Dooley et al. [1] has estimated that resupply of even a first-generation ALS will be limited to 15% of the system mass. The remaining 85% must be perpetually cycled through the life support system

NASA scientists and engineers will need to feel confident that the proposed ALS components will perform as designed and will operate as expected. Unreliable components and processes that threaten crew life and mission success should be eliminated from consideration during early stages of ALS system design. Furthermore, the external environment in space and on planetary surfaces will be hazardous to crew, crops, and other organisms. The hazards will include low temperatures and pressures, harmful radiation, and potentially toxic chemicals. Reliable, stable ALS components and processes with low rates of failure in these conditions and will ensure crew safety in the midst of these hazards.

Stability (of a process) is often included in the concept of component or system reliability. A good characterization of a stable bioprocess is given by Bailey and Ollis [2]. A stable process will not likely change or be affected adversely by a variation in environmental or operational conditions. A stable process will return to steady state equilibrium or to the original performance level after having been perturbed. A stable process will be resistant to change. All of these characteristics of stability are desired in ALS components or processes.

Since 1989 our resource recovery research at the Kennedy Space Center (KSC)-ALS Breadboard Project has focused on microbial-community based components for bioprocessing solid wastes--notably inedible crop residues [3, 4, 5]. The majority of these studies have used continuous stirred tank reactors (CSTRs). Studies on the effects of environmental and process parameters using CSTRs were usually of short duration, 1 to 4 weeks. Carbon dioxide and bioreactor effluents, produced during longer CSTR runs of 70 to 418 days, were successfully used for partial replenishment of crop hydroponic nutrient solutions at both intermediate (ca. 0.5 to 1 m^2) and breadboard scale (5 to 10 m^2) crop growing area. Data from these studies were selected as examples of biological reliability and stability for this paper.

In this paper we show the reliability that was possible with CSTRs if environmental and process variables were kept reasonably constant and unvarying. These examples of biological reliability were selected from runs at two extremes of operating conditions - slow and fast retention times. We then present examples that demonstrate the stability of bioreactors when environmental and process variables were NOT kept constant and unvarying. We present results from unplanned perturbations to three environmental parameters--pH, dissolved oxygen, and temperature--and of one planned perturbation to the nutritional status of the microbial community by starving them for periods ranging between 2 and 10 days. These examples of biological stability do not include conditions that are outside of the "tolerance range" of living microorganisms

for that variable because our original bioreactor design criteria were selected to stay within these limits.

MATERIALS AND METHODS

BIOREACTOR EQUIPMENT AND CONDITIONS - The Breadboard Project bioreactors for resource recovery were designed and fabricated in two sizes: an intermediate-scale (8 liters working volume) and a breadboard-scale (120 liters working volume). These sizes were selected so bioreactor effluents could be used to replenish hydroponic solutions for Integrated resource recovery and crop growth studies. Integrated studies were also conducted at a bench scale (0.5 to 1 m^2) and breadboard scale (20 m^2) plant growing areas in environmental chambers and in the KSC Biomass Production Chamber (BPC). A detailed description of the design and operation of these two bioreactor sizes has been published [3, 4, 5].

Environmental Conditions - Bioreactor environmental variables were kept constant. Bioreactor pH was computer controlled at a set point of 6.5 by addition of 1 N nitric acid. Temperature was maintained at 35°C by computer actuation of a solenoid valve that controlled the flow of 45°C water from an external circulating water bath though insulated stainless steel coils wrapped around the bioreactor. Dissolved oxygen was kept above 2.0 mg L^{-1} by an air flow that ranged between 7.0 and 7.5 liters min^{-1} and a stirring rate of 320 to 360 rpm.

Bioreactor Process Conditions - Process variables were also controlled. Substrate concentration was usually 20 gdw crop residue $liter^{-1}$. Bioreactor retention time was varied by adjusting the bioreactor harvest interval according to specific experimental protocols and crop growth requirements. Bench-scale and breadboard scale studies used either wheat residues (cultivar Yecora rojo) or potato residues (cultivar Norland), which were grown under controlled conditions in the BPC. These residues were either oven-dried (70°C until dry) or freeze dried, then milled to 2 mm diameter. Milled inedible biomass was introduced into bioreactors by a screw auger located in the bottom of a stainless-steel feed hopper. The auger conveyed solids out of the hopper and into the bioreactor just above the liquid level. The volumetric feed rate for dried crop inedible biomass was variable.

Bioreactors were inoculated with a commercial source (Digestase FDE 750, Tech-Line Products, Milwaukee, WI) in addition to the microflora native to the crop residues and de-ionized water source.

ANALYTICAL METHODS - Offgas measurements - CO_2 concentration in the bioreactor exit gas (offgas) was measured with an infra-red CO_2 analyzer (Licor Model LI 6252).

Computer Monitoring - Dissolved oxygen, pH, temperature, gas flow rate, and offgas CO_2 concentration were monitored continuously as described by Finger and Strayer [3]. Hardware for monitoring and control included a SUN Sparc Station and OPTO-22 digital and analog input/output boards. Software (UNDACE V1.9) was developed at KSC for monitoring and control of ALS breadboard components and was also the primary information interface between the operator and the bench-scale bioreactors (6). All monitored parameters were average over 5 or 10 minute intervals and archived on a Hewlett Packard Model 9000 I-50 mainframe computer.

RESULTS AND DISCUSSION

BIOLOGICAL RELIABILITY - To demonstrate biological reliability during processing of crop residues we present results of bioreactors operated at two extremes of retention time.

A long duration, retention time of 48 days during breadboard-scale run of 418 days - The Breadboard-Scale Aerobic Bioreactor (B-SAB) was continuously operated for 418 days to supply nutrient replenishment solution (filtered bioreactor effluent) and carbon dioxide to hydroponically grown white potato (10 m^2 growing area in the BPC. The effluent output from the B-SAB had to be "throttled" to match the variable nutrient demands of the potato crop in the BPC throughout the 4 sequential crop growth cycles (105 days each cycle). Throttling was accomplished by altering the bioreactor retention time between 24 and 48 days. Figure 1 shows B-SAB reliability over an 8 week period of operation (48 day retention time) during which environmental and process (i.e., retention time) conditions were constant and unvarying. The average offgas CO_2 concentration, measured at 5 minute intervals for 52 days of operation is plotted. Four bioreactor harvest days during the 8 weeks were not included in the Figure 1 averages. The 95% confidence limits show the reproducibility of this daily CO_2 production curve and clearly indicate the reliability of the biodegradative process during constant, unvarying conditions.

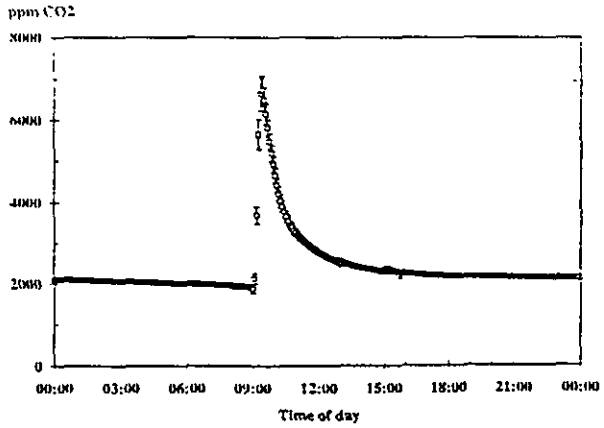

Figure 1. Average offgas CO_2 concentration (ppm) measured at 5 minute intervals from midnight to midnight during operation of the KSC-Breadboard-Scale Aerobic Bioreactor. Operation was for 52 days . excluding 4 bioreactor harvest days. Retention time: 48 days, feeding rate: 62.5 gdw day^{-1} , pH: 6.5, temperature: 35°C, aeration rate: 7.5 L min^{-1} , stirring rate: 325 rpm. Error bars are 95% confidence limits.

Retention time of 0.25 days during a bench scale run - This example of biological reliability was taken from the other extreme for retention time that we have tested at KSC. As part of a study to determine the lower limit for retention time, we sequentially lowered the retention time from 2.0 to 0.25 days, allowing the bioreactors to reach steady state at each retention time. Once steady state was reached, the response variables were required to be constant for at least 3 times the retention time before collection of effluent samples and changing the retention time to test the next, shorter retention time. We anticipated that the bioreactor performance might suffer and become "unstable" at these shorter retention times.

Figure 2 demonstrates the biological reliability of a bench scale CSTR operating at a retention time of 0.25 days (6 hr.). The CO_2 production peaks occurred over each harvest period of 3 hours. Feeding of the bioreactor was continuous and the feeding rate was adjusted to ensure that all of the feed material was added before the next harvest (i.e., the CO_2 production decreased each harvest period as the feeder ran out of material to add).

Although the shape of the CO_2 production curves for each of the seven harvest periods shown were not identical, the total amount of CO_2 produced per each harvest period (i.e., sum of CO_2 produced per 3 hour period) was similar (upper curve, Figure 2), with low variability (average 384 +/- 8.4 mL CO_2 [i.e., coefficient of variation, CV, of 2.2%]). This reproducibility of microbial respiration over constant, unvarying harvest periods demonstrates biological reliability at this fast retention time.

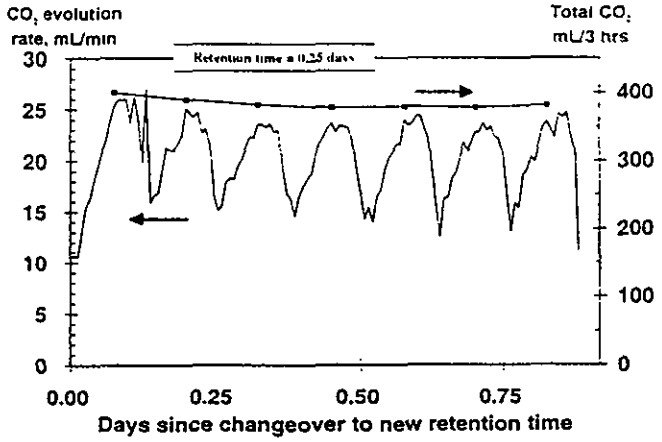

Figure 2. *Reliability of microbial community respiration during biodegradation of inedible wheat residues in a bench scale CSTR (8 liter working volume) with the fastest retention time tested during KSC studies. Plotted are CO_2 evolution rate calculated at 10 minute intervals and total CO_2 produced per harvest period of 3 hours. pH: 6.5, temperature: 35°C, retention time: 0.25 days, operating volume: 4 liters, feeding rate: 160 gdw/3 hour harvest period, aeration rate: 7 Lpm, stirring rate: ca. 320 rpm.*

BIOLOGICAL STABILITY - *"If a steady state is stable, the system will return to that steady state after a small disturbance has acted and moved the system slightly away from the steady state of interest. For an unstable steady state, the biomass, substrate, and other concentrations will 'run away' from their steady state values following a small disturbance."*--Bailey and Ollis [2].

We have found that the production of carbon dioxide by the CSTR microbial community is a useful response variable for indicating bioreactor performance. Carbon dioxide, and water are the major products of microbial respiration. The respiration rate is linked to the amount of microbial biomass present in the bioreactor and to the rate of microbial growth under aerobic conditions [7, 8, 9]. Carbon dioxide in the bioreactor offgas is easily measured on-line in real time (CO_2 specific infrared Gas Analyzer--IRGA), which makes it useful for determining the effects of both planned and unplanned disturbances (or perturbations as we call them) to the microbial community. We used on-line bioreactor CO_2 production to monitor the effects of perturbations on the stability of the bioreactor performance or steady state.

Examination of CSTR performance in the KSC-ALS breadboard with unplanned perturbations found a number of disturbances caused by hardware and software failures and operator errors. Since July of 1994 there have been 78 such perturbations, mostly with minor consequences to bioreactor stability. Level of pH below the setpoint of 6.5 were responsible for 22 incidents. Low temperature (ambient compared with the setpoint of 35°C) caused 21 incidents and high temperatures of 44°C caused 2 potentially harmful incidents. Low dissolved oxygen levels, caused by disruption of airflow or stirrer malfunction, resulted in 15 perturbations. Excessive foaming caused both low pH and low DO, and caused 13 of the perturbations, but occurred mostly at the start-up of bioreactor runs when steady states had not been achieved. Two of the more serious CSTR perturbations were a result of multiple problems caused by a failure of the UNDACE computer monitoring and control system. Last, three unplanned perturbations were caused by biomass feeder malfunction, i.e., the microbes were starved/deprived of nutrients.

The most severe examples resulting from low pH, high temperature, and low DO are presented. We conclude the stability portion of this paper with results from a planned perturbation--the intentional starvation of a CSTR during long term operation for replenishment of crop nutrients.

Effects of unplanned pH perturbation - In the absence of pH control, aerobic decomposition of ALS crop residues will result in a pH increase to 8.3 or higher with a concomitant decrease in soluble nitrate (unpublished results). To keep microbial decomposition at optimal levels, we desired to control pH in the middle of the tolerance range of most microbes--around 6.5 to 7.0. We prevented the pH in aerobic CSTRs from going above a setpoint of 6.5 by computer controlled addition of 1 N nitric acid.

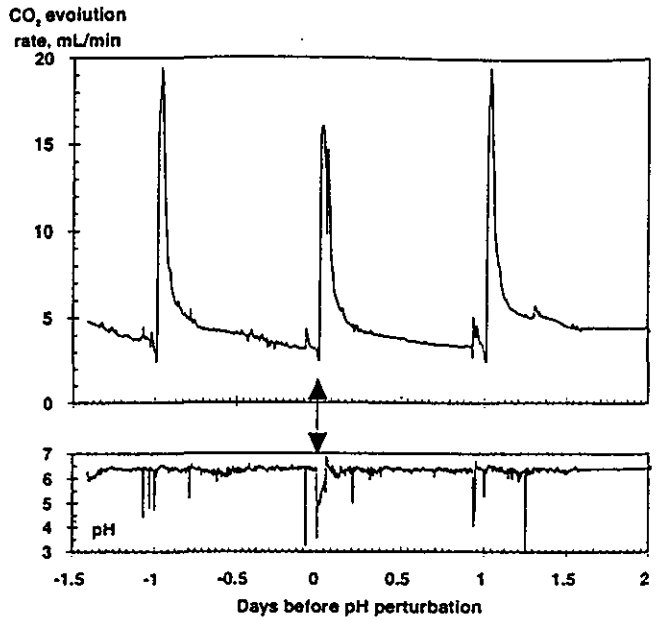

Figure 3. *Effects of an unplanned pH perturbation on CSTR respiration for 1 day before and 2 days after the disturbance, which occurred at day 0 of this plot. Top panel: CO_2 evolution rate, bottom panel: pH. The CSTR run was part of a study of the effects of retention time on bioprocessing. CSTR working volume: 8 L, retention time: 10.67 days, feeding mode: pulse, feeding rate: 15 gdw (wheat residues) day^{-1}, pH: 6.5, temperature: 35°C, aeration rate: 7.0 L min^{-1}, stirring rate: 320 rpm.*

Unplanned pH perturbations were most often the result of excessive foam, which prevented the added 1 N nitric acid from reaching the bulk solution and, thus, lowering the pH detected by the pH probe. The presence of foam resulted in an overshoot of acid addition, which ultimately lead to a pH drop of several units below the setpoint when the acid finally reached the bulk solution through the foam.

The results of the worst acid addition incident at KSC since 1994 is presented in Figure 3. The CSTR run affected was being used to study of the effects of bioreactor retention time on crop residue biodegradation. An acid addition overshoot (time zero) occurred 25 minutes before the bioreactor was harvested and fed for the day and resulted in a lowering of the pH to 3.6 for 5 minutes, followed by 30 minutes at pH 5.0, and 45 minutes at pH 5.5, when the operator added base to bring the pH to 6.5.

The maximum CO_2 evolution rate, which typically occurred shortly after feeding, was decreased by 18% by the pH disturbance, when compared with the maximum rate from the previous day. This rate returned to normal (identical to the peak for the day prior to the perturbation) the day following the pH perturbation. The total CO_2 produced for the entire day was 96% of the total for the previous day and this parameter returned to normal the day following the perturbation.

According to the definition for process stability that we gave in the introduction to this section, the microbial decomposition of crop residues in a CSTR was stable to a moderate variation in pH (3 log scale concentration). Respiration was affected by the perturbation but returned to original performance following the displacement.

Effects of unplanned dissolved oxygen perturbation - Oxygen, of course, is a key substrate in aerobic bioreactors. The lack of adequate dissolved oxygen levels can drastically change microbial growth rates, activities, and products of metabolism. [1, 8, 9]. CSTR dissolved oxygen (DO) levels were controlled by manipulating the air flow and the stirring rate. Most perturbations to DO were caused by interruption in the air flow. However, air flow problems were of short duration with operator action taken to correct the problems. Often the drop in DO was not enough to cause a significant change in respiration rate. During many CSTR runs, daily DO drops below 1.0 mg L^{-1} lasted for up to 30 minutes immediately after pulse feeding. When unplanned DO perturbations occurred, the community, already adapted to fluctuating DO, recovered rapidly.

The most severe low DO perturbation was caused by an 8 hour stirrer failure and is shown in Figure 4. Because air flow was unaffected, the DO did not plummet below 1.0 mg L^{-1}, but showed a slow decline. When the problem was noticed and corrected prior to the daily pulse feeding, the DO returned to pre-incident levels. The

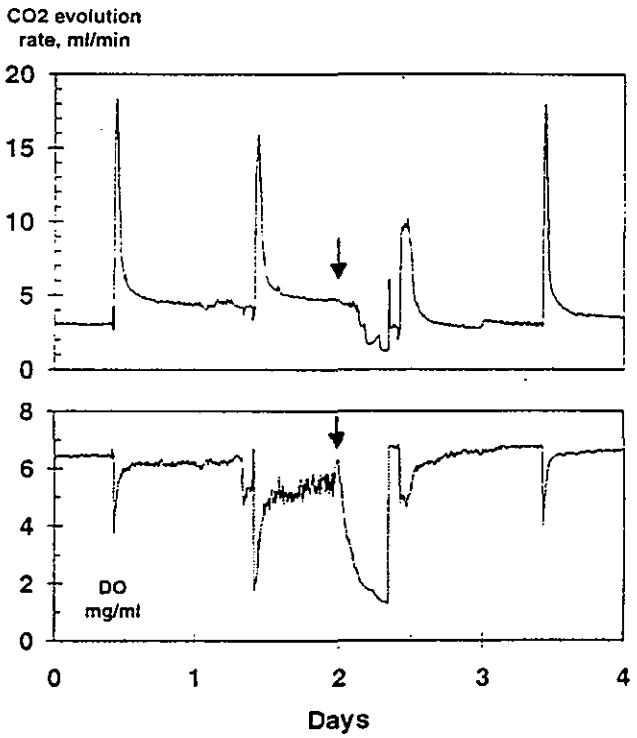

Figure 4. *Effects of an unplanned dissolved oxygen perturbation on CSTR respiration. Top panel: CO_2 evolution rate, bottom panel: D.O. The CSTR run was part of a study of the effects of retention time on bioprocessing. CSTR working volume: 8 L, retention time: 5.33 days, feeding mode: pulse, feeding rate: 30 gdw (wheat residues) day^{-1}, pH: 6.5, temperature: 35°C, aeration rate: 7.0 L min^{-1}, stirring rate: 320 rpm.*

response of the respiring microbial community to this perturbation was similar to the pH perturbation shown in Figure 3. The CO_2 evolution peak for the day of the incident was adversely affected, but respiration returned to normal the following day.

These results again show the stability of CSTR biological processes to a low level, short duration perturbation. CSTR respiration was stable to a moderately severe variation in dissolved oxygen. Respiration was adversely affected by the perturbation but returned to original performance following the displacement.

Effects of unplanned temperature perturbation - As with the other environmental parameters discussed in this paper, the response of a mixed microbial community to temperature extremes would be characteristic of the mixture of microbes that make up the community. All microorganisms have a characteristic optimal growth temperature (or pH or dissolved oxygen concentration) at which growth and reproduction rates would be highest. Microorganisms also have minimal growth temperatures below which they are metabolically inactive and upper limits beyond which they fail to grow [10].

There were a number of unplanned temperature perturbations to CSTRs, but nearly all were caused by failure to maintain the water level of the circulating water bath. This error usually resulted in CSTR temperature falling from the 35°C setpoint to ambient (~ 25°C).

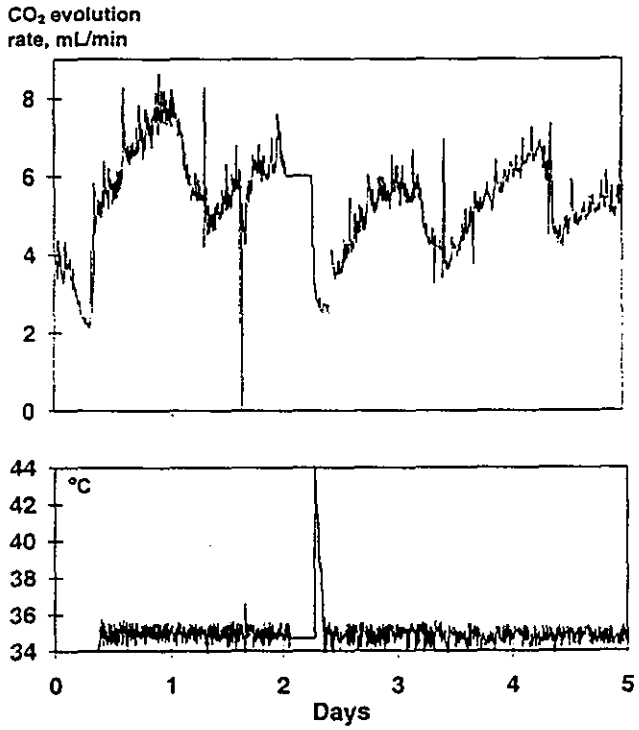

Figure 5. Effects of an unplanned temperature perturbation on CSTR respiration. Top panel: CO_2 evolution rate, bottom panel: Temperature. The CSTR run was part of a study of the effects of very rapid retention times on bioprocessing. CSTR working volume: 4 L, retention time: 2.00 days, feeding mode: continuous, feeding rate: 20 gdw (potato residues) day^{-1}, pH: 6.5, temperature: 35°C, aeration rate: 7.0 L min^{-1}, stirring rate: 320 rpm.

Respiration rates were lower during these incidents, but recovery was rapid once bioreactor temperature was brought back to the setpoint.

In two instances the temperature increased above the setpoint following a failure of the computer control and monitoring system (UNDACE). These control failures occurred when the solenoid valve from the recirculating water bath to the bioreactor was in the open position. For the example shown in Figure 5, the failure occurred at midnight (Day 2) and lasted for about 8 hours. The CSTR operating temperature increased to 44°C, just below the 45°C setpoint of the circulating water bath. The length of CSTR exposure to 44°C is unknown because data collection was also disabled during the control system outage (flat line portion of both temperature and CO_2 evolution during Day 2 in Figure 5).

The shape of the daily CO_2 evolution curves in Figure 5 are different from those in Figures 3 and 4 because the mode of biomass feeding was different. Pulse feeding of ca. 30 minutes duration was used for the CSTR studies shown in Figures 3 and 4. During the rapid retention time example shown in Figure 5, CSTR feeding was nearly continuous as we were concerned that pulse feeding would adversely affect DO levels. The shape of the CO_2 evolution curves in Figure 5 reflect continuous feeding.

Nonetheless, the high temperature incident caused a 26% reduction in total daily CO_2 production following the perturbation and 17% for the next day. CSTR recovery beyond two days (day 5) could not be determined because of other CSTR operational problems unrelated to the temperature incident. The temperature perturbation had a dramatic effect on microbial community respiration, but a slow recovery to pre-incident levels was probably in progress.

The 44°C temperature was within the tolerance range of most mesophilic bacteria, but some metabolic functions and/or some microbial populations may have been lost during the exposure period. Because recovery might have been incomplete, the CSTR microbial community may not be stable to exposure to sublethal temperatures, but the CO_2 production indicates functional recovery.

Effects of planned starvation periods (nutrient perturbation) - Since 1994 , a few unplanned incidents of CSTR starvation have also occurred during bioreactor operation. These events were usually caused by operator error, namely failure to properly seal the biomass feed hopper after filling. A portion of the moisture laden bioreactor offgas then exited the bioreactor through the feed hopper and wetted the crop residue biomass. Much of the wet biomass was retained in the hopper and not fed into the bioreactor via the auger.

We observed that an unfed bioreactor recovered from these one day starvation periods during the next feeding (data not shown). One of the co-authors (MPA) noted that this apparent bioprocess stability could be used to advantage during long duration CSTR/crop production integration studies. By starving the bioreactor over weekends and during periods when bioreactor effluent

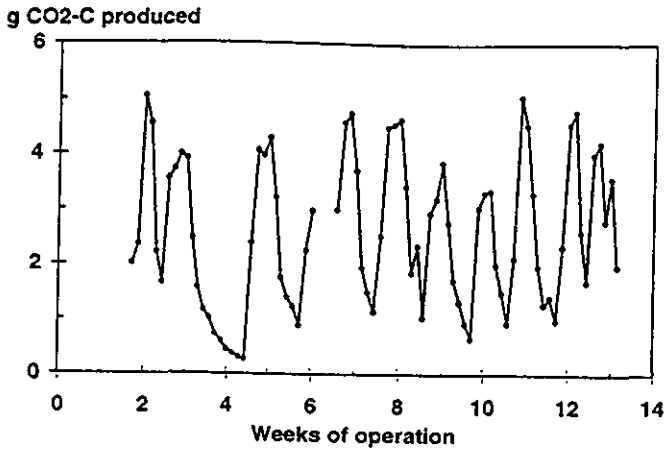

Figure 6. Effects of intentional starvation (nutrient perturbation) on CSTR respiration. Plotted is the daily total CO_2 produced each day for the 13 week duration of the study. The CSTR run was part of an integrated KSC study with the bioreactor effluent used to partially replenish a crop hydroponic nutrient solution. CSTR working volume: 4 L, retention time: 1.00 days, feeding mode: continuous, feeding rate: 20 gdw (potato residues) day^{-1}, pH: 6.5, temperature: 35°C, aeration rate: 7.0 L min^{-1}, stirring rate: 320 rpm.

output exceeded crop nutrient demand, reductions in biomass processed and operational manpower could be realized.

The effects of nutrient deprivation on microbial community respiration in a CSTR are shown in Figure 6. This CSTR run was part of an integrated resource recovery/crop production study. The retention time for the CSTR, when fed, was 1 day. The CSTR was subjected to various periods of intentional starvation, ranging between 2 and 10 days. Feeding periods after starvation were usually for 5 consecutive days, but, when crop nutrient demands were low the number of consecutive feeding days were lower.

The response of the CSTR microbial community to each starvation period was an exponential decline in respiration rate. Community respiration usually returned to normal levels within two days after feeding was resumed. These results again demonstrate biological stability of the CSTR following a perturbation.

"Steady state" respiration rates during feeding ranged between 4 and 5 g CO_2-C day^{-1}. A concomitant run of a control, unstarved CSTR was not possible during this study. Comparison with a previous, unstarved CSTR run, with similar values for environmental and process parameters, indicated that the daily "steady state" CO_2 production was decreased by 29% (~4.5 vs. 6.3 g CO_2-C day^{-1}). Although the starvation perturbation did not cause a "run away" response, it did significantly reduce performance in terms of respired CO_2.

The periodic feeding and starving of our CSTRs is similar in concept to an emerging bioreactor technology called sequencing batch reactors. Periodic unsteady state processes, such as fluctuating feed streams, are being used in controlled bioreactors to process wastes. The entire issue #1 of Water Science and Technology was devoted to this topic [11, 12].

CONCLUSION

The reliability of continuous stirred tank reactors for bioprocessing ALS solid wastes (crop residues) has been demonstrated by integrated resource recovery and crop production studies at the KSC Breadboard Project. Bioreactor performance was reliable over long periods (52 days duration at constant input and operational parameters) as well as at extremes of process conditions, such as retention time (0.25 days and 48 days).

When bioreactor processing conditions were variable, or not constant, the stability of the bioprocesses to return to steady state conditions has been demonstrated for pH, DO, and low temperature. Planned perturbations to feeding regimes also demonstrated bioprocess stability.

For all three unplanned perturbation types--low pH, low DO, and high temperature--further research will be needed to better elucidate their effects on bioprocessing stability. Further research is suggested because our most extreme example of pH variation (unplanned) was of moderate nature and short duration. Microorganisms generally cannot tolerate extreme pH values [1, 9, 8], so we need to design experiments to examine CSTR biological stability following an intentional disruption of lower (or higher) pH that lasts for a longer exposure period. As with pH, further research with a controlled DO perturbation of known intensity and duration is suggested to better understand CSTR stability.

Although the observations presented here suggest that bioprocessing of crop residues is stable to minor perturbations, well designed perturbation studies would provide the data needed to reach a definitive conclusion regarding bioprocess stability. Most of the unplanned perturbations we have observed at KSC have fallen well within the known tolerance range of bacterial processes. In general, this reflects our initial design criteria for the CSTRs--to be controlled within these limits. It also demonstrates that biological processors benefit from the fact that life is tenacious, flexible, and persistent - all highly desired attributes leading to stability and reliability in an advanced regenerative life support system.

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